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Possible mechanisms behind cardiac troponin elevations

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ABSTRACT
Cardiac-specific troponins are elevated in blood following cardiac injury and are the preferred diagnostic biomarkers when acute myocardial infarction is suspected clinically. Cardiac troponin (cTn) elevations are also observed in clinical conditions without obvious connection to cardiac injury. Irrespective of the underlying condition, cTn elevation is linked to a poor prognosis, even if the elevation is stable over time. Here, we explore mechanisms that may lead to cTn elevations, including necrosis, apoptosis, necroptosis, cell wounds and decreased clearance. The aim is to broaden the perspective of how we interpret unexpected cTn elevations in patients. The cTn elevations may not be able to serve as direct proof of myocardial necrosis especially in the absence of a clear-cut reason for its release.

Abbreviations:
AMI: acute myocardial infarction; cTn: cardiac troponin; cTnI: cardiac troponin I; cTnT: cardiac troponin T; MLKL: mixed lineage kinase domain-like; TUNEL: terminal deoxynucleotidyl transferase nick end labeling

Introduction
Cardiac troponin I (cTnI) and cardiac troponin T (cTnT)) are cardiac-specific proteins that are part of the tropinin complex of thin filaments which form together with thick filaments the contractile apparatus, the sarcomere, of cardiomyocytes (Figure 1). Cardiac troponin (cTn) is released and is transiently increased in the blood following an acute myocardial infarction (AMI) and other types of acute myocardial injury. The cTns are the preferred biomarkers for the laboratory diagnosis of AMI [Roffi et al. 2016], due to their excellent cardiac specificities, the fact that healthy individuals have only low levels [Cardinaels et al. 2012], and the fact that there are excellent assays available for clinical use.

The cTn elevations are also found in patients with chronic cardiac comorbidities. Recently, increases have been observed in normal individuals in response to what might be considered physiological stress such as exercise, rapid atrial pacing and after the infusion of dobutamine. A large number of studies have shown in non-AMI populations that elevation of the cTn concentration, including some not above the 99th% upper reference limit, is a strong risk marker for death, the development of heart failure and ischemic heart disease and other non-ischemic cardiac diseases [Galvani et al. 1997, Aviles et al. 2002, Peacock et al. 2008, Omland et al. 2009, Kawahara et al. 2011, Aldous et al. 2015, Omland et al. 2015, Roos et al. 2017]. At present, we only have evidence-based treatment for patients who have had AMI. For other conditions associated with cTn elevations, treatments are frequently advocated based on the assumed pathophysiology but data supporting their effectiveness are not available [Roos et al. 2017].

Herein, we summarize what is known about the possible cellular mechanisms behind cTn release and clearance, which may broaden the perspective on how to interpret cTn values in clinical practice. In most cases, information is available for both cTnT and cTnI (cTn), but in some instances, information is only known for cTnT or cTnI and in those situations, that fact will be made clear.

CTn release by necrosis
The most obvious reason for cTn elevation in a patient, especially if the cTn elevation is transient, is necrosis. In fact, compared with other cell types, cardiomyocytes are more prone to undergo necrosis triggered by situations such as the Ca2⁺ paradox and/or the oxygen paradox [Piper 2000].

Myofibrils, which constitute 50–60% of the cardiomyocyte volume, are repeating units of Ca2⁺-activated, ATP-consuming sarcomeres (Figure 1). Therefore, if Ca2⁺ leaks into the cardiomyocyte cytoplasm, the sarcomeres contract and

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quickly consume all the ATP (Figure 2(A)), resulting in rapid necrosis and release of cTn along with other cellular contents (Piper et al. 2003).

A visual representation of the rapid nature of cardiac necrosis is the ‘stone heart syndrome’ after the Ca\textsuperscript{2+} paradox. If isolated hearts are first perfused with a buffer without, and then a buffer with Ca\textsuperscript{2+}, the entire heart contracts tonically, turning the perfusion buffer red due to the release of myoglobin and the heart becomes a white hard contracting mass of necrotic cardiomyocytes called a ‘stone heart’ (Piper 2000).

Groundbreaking studies by Jennings showed that ischaemic cardiomyocytes quickly develop intracellular oedema when ATP levels drop (Jennings et al. 1978). As the sarcomere is anchored in the plasma membrane via the dystrophin protein complex, swelling does not result in uniform enlargement of the cardiomyocyte; rather, membrane ‘blebs’ (Schwartz et al. 1984) are formed between the dystrophin protein complex anchoring points (Jennings et al. 1978, Sage and Jennings 1988) (Figure 3). In this situation, the cardiomyocyte plasma membrane becomes fragile but does not break immediately (Jennings et al. 1983), unless exposed to an acidic or hyposmotic environment (Jennings et al. 1983, Steenbergen et al. 1985). However, when contractility occurs after reoxygenation (Vander Heide and Ganote 1987), or when anoxic cardiomyocytes are forced to move by surrounding non-ischaemic cardiomyocytes (Steenbergen et al. 1985), or if the ischaemic heart is acutely diluted by a balloon in the ventricular chamber (Vander Heide and Ganote 1987), these cells will succumb and result in contraction band necrosis.

Some of this damage can be mitigated by a reduction in contractile performance, so called stunning. Myocyte contraction stops within a few heartbeats following total ischaemia in what is thought to be a mechanism that protects oedematous ischaemic cardiomyocytes from contraction-mediated
cell damage, termed the ‘smart heart hypothesis’ (St Louis et al. 2000, Depre and Vatner 2005). If the resulting membrane damage allows enough Ca\(^{2+}\) to enter the cardiomyocyte cytoplasm, the sarcomeres contract tonically and consume what little ATP is left, limiting the ability of the cardiomyocytes to pump out excess Ca\(^{2+}\). In the ATP-depleted and Ca\(^{2+}\)-loaded state, myofibrillar shortening stays fixed in all the sarcomeres, as the cross-bridges between actin and myosin remain in an attached state (Piper et al. 2003), resulting in contraction band necrosis where collapsed sarcomeres and large membrane defects are seen in histological preparations (Figure 3; Jennings 2013). Unfortunately, much of the literature concerning cardiomyocyte necrosis is substantially dated and probably need to be revisited with newer techniques. In addition, during the past 15 years, this simple ‘necrosis by breaking’ picture has been complicated by the realization that programmed necrosis – necroptosis, described below – also operates in the ischaemically damaged heart (Kung et al. 2011). In essence, the possibility of programmed necrosis occurring at a later stage further complicates the interpretation of the cTn release kinetics following AMI and other myocardial injury. Nonetheless, in this acute situation, it is thought that most cTn release is due to irreversible cellular injury.

**cTn release mechanisms after membrane damage**

The cTn binds with finite affinity to thin filaments of the sarcomere (Shiraishi et al. 1992) (Figure 1(C)). Although release is immediate after membrane damage, the washout of cTn is slowed when compared to other cardiac injury biomarkers due to what has been called ‘the trapping effect’ (Starnberg et al. 2014). This is especially true when there are large volumes of the myocardium that become necrotic, such as after a transmural AMI. These types of insults result in sustained elevations of cTn for days and sometimes weeks (Katus et al. 1991, Laugaudin et al. 2016). The delayed washout of cTn is in contrast to that of cytoplasmic cardiac injury biomarkers (Figure 4), such as lactate dehydrogenase (LDH), creatine kinase (CK) (Katus et al. 1989), CKMB (Katus et al. 1987) and myoglobin (Sylven and Bendz 1978), which have no affinity for cardiac tissue. Thus, if cardiac tissue is crushed in warm plasma, myoglobin and cTn are released immediately but the washout of cTn takes more time, due to its binding to the insoluble sarcomere. Starting within a few hours, the necrotic cardiac tissue induces local inflammation and accumulation of neutrophils and macrophages that digest damaged tissue, forming granulation tissue and then a stable scar (Pfeffer and Braunwald 1990). During this later process, cTn release is likely, largely due to degradation of myofibrils. Many of these other proteins, such as CK, are locally degraded and not detected by conventional clinical assays. Consequently, once blood flow is re-established, they no longer can be appreciated (Clark et al. 1978).

As in all binding reactions, the washout of cTn is volume dependent and, hence, is faster and more complete if the plasma volume or blood flow is capacious (Starnberg et al. 2014). Increases in cTn levels hours or days after the initial non-reperfused AMI can, in addition to re-infarction, be due to partial restoration of flow (Hermens et al. 2015). Also, if cardiomyocyte necrosis is scattered, as observed after isoproterenol-induced type II infarctions in rats, the washout of cTn from necrotic myocytes could, in principle, be over within 24 hours (York et al. 2007, Zhang et al. 2008, Clements et al. 2010). The cTn elevations that are cleared within 24 hours thus still can be due to cardiomyocyte necrosis.

**cTn release by apoptosis and other regulated cell death pathways**

All cells, including cardiomyocytes, have built-in ATP-consuming death programmes that are activated when appropriate signals are present. This process is essential for the removal of interdigitating cells in the formation of fingers during embryogenesis (Svandova et al. 2017). However, apoptosis and other death programmes are probably most important in the defence against intracellular parasites like viruses.

Mammalian cells have evolved an array of mechanisms to detect the presence of intracellular pathogens, with many of these pathways culminating in the activation of a cell death response. Some of these responses were found through studies of the cytomegalovirus (CMV) that harbours several genes essential for viral replication dedicated to block apoptosis and other cell death programmes. Inhibition of cell death not only affords CMV an opportunity to complete the replication cycle, but also restricts the release of cellular content and, hence, limits the inflammatory response required for the generation of the adaptive immune response (Brune and Andoniou 2017).

In the unlocking of the multitude of signals that can activate programmed cell death, including TNF receptor activation (Guo et al. 2017), it has become evident that our cells are like primed rat traps, eager to go off at any sign of invasion, but – perhaps not intentionally – also in response to oxidative overload, ischaemia and other stressors (Brune and Andoniou 2017).

In principle, using its original description by Kerr (Kerr et al. 1972) (Figure 2(B)) and the latest definition of apoptosis by the Nomenclature Committee on Cell Death (NCCD) (Galluzzi et al. 2012), apoptosis should not result in cTn elevations. In fact, it has been suggested that apoptosis cannot

![Figure 4. Kinetics of cardiac troponin T (cTnT), creatine kinase (CK) and lactate dehydrogenase (LDH) from the first published patient with acute myocardial infarction where a cTnT assay was used by Katus (Katus et al. 1989).](image)
be the culprit if the patient has cTn elevations (Takemura et al. 2013). This is because no intracellular content is expected to be released when the apoptotic cell is divided into membrane-enclosed apoptotic bodies with phosphatidylserine and other ‘eat-me’ signals exposed on the surface, resulting in the engulfment and lysosomal degradation of the apoptotic bodies by surrounding cells and sometimes by immune cells (Nagata et al. 2010).

It is, however, possible that the myofibrils in cardiomyocytes interfere with the formation of tight membrane-enclosed apoptotic bodies, or that apoptotic bodies break and expel their content before they are cleared (Figure 2(B)). It is also possible that other forms of programmed cell death operate, like necroptosis, which ultimately results in lysis of the cell after the formation of multimers of the protein mixed lineage kinase domain-like (MLKL) that bind to and make holes in cellular membranes (Kung et al. 2011) (Figure 2(C)). Although never reported, cardiomyocytes undergoing necroptosis most likely also release cTn as the nuclear protein HMGB1 is released in the process. Our experience is that cTn release from damaged primary cardiomyocytes always overlaps with the release of other intracellular proteins, like LDH and myoglobin (Starnberg et al. 2014).

Unfortunately, many studies of apoptosis in the human heart following AMI have methodological problems (Takemura et al. 2013). In addition, most studies are performed on hearts from dead patients, possibly adding a selection bias and effects secondary to the cause of death (Abbate et al. 2003). Sometimes, studies have neglected the fact that only 30% of the cells in the normal heart are cardiomyocytes and even less in granulation tissue, because of accumulation of apoptosis-prone immune cells (Saraste et al. 1997, Wakabayashi et al. 2015, Jose Corbalan et al. 2016). In addition, no attempt was made to relate the extent of apparent apoptosis to the cTn levels in these human studies. Consequently, we do not know if, or to what extent, apoptosis contributes to acute or stable cTn elevations in humans. However, increased levels of caspase-3, thought to be a mediator of apoptosis, are found systemically in patients with AMI (Agosto et al. 2011).

What is clear, however, is that the cells in human hearts seem to undergo some kind of apoptosis-like process during an extended period following AMI, also in areas not affected by ischaemia (Abbate et al. 2008), possibly involved in the thinning of the remote myocardium that is often observed after a large MI (Pfeffer and Braunwald 1990). Similar apoptosis-like processes have been found in human biopsy studies of other cardiac pathologies like heart failure (Narula et al. 1999). In these studies, cardiocytes display several hallmarks of apoptosis, including terminal deoxynucleotidyl transferase nick end labeling (TUNEL)-positive nuclei and caspase activation co-localizing in cells expressing myocyte-specific proteins that correlate with cardiac disease and outcome (Abbate et al. 2003, Biondi-Zoccai et al. 2004). Some authors have faith in methods to detect apoptosis not involving electron microscopy (Saraste and Pullki 2000). However, according to other authors, TUNEL and other DNA-end labelling methods cannot unequivocally prove that apoptosis has occurred. This controversy has led to some cases where the interpretation of the published results has been questioned because of discrepancies between the methods (Takemura et al. 2013).

There is a large body of studies in mice where cardiomyocyte apoptosis or necroptosis can be induced by genetic manipulation, like limited induction of the apoptosis-inducing protease caspase-8, which has been shown to result in the development of heart failure (Wencker et al. 2003) and larger infarcts after coronary occlusion. If the apoptotic or necrotic programme is inhibited by genetic or pharmacological means, the almost uniform finding is that the size of experimental infarcts is reduced (Yaoita et al. 1998, Holly et al. 1999, Hochhauser et al. 2003, Abbate et al. 2008) and mice are protected from the development of heart failure (Wencker et al. 2003,Kitamura et al. 2014, Wang et al. 2014). There are a few studies reporting apoptosis following ischaemic challenges in larger animals (Goussev et al. 1998). One study has shown a correlation between sustained cTnI elevations and the amount of TUNEL-positive cells in pigs after brief periods of ischaemia (Weil et al. 2017).

At this stage, it is safe to conclude that some type of programmed cell death operates in the heart and that necroptosis could contribute to cTn elevations in patients; however, the current data are not sufficient to make strong statements about how programmed cell death influences cTn elevations more chronically in patients. However, it is fair to speculate that increases in myocardial stress due to volume expansion (Feng et al. 2001) and potentially subendocardial ischaemia due to supply–demand imbalance or transient increases in pulmonary pressures could result in activation of these pathways with cTn release. Such speculation would fit with the release of cTn shown to occur in normal subjects who are rapidly atrially paced (Turer et al. 2011) and/or exposed to dobutamine (Siriwardena et al. 2012). However, other non-apoptotic mechanisms also could explain these findings.

**cTn release by cell wounds**

Apparently viable cardiomyocytes in Petri dishes or in animal models exchange macromolecules over their plasma membranes (Cooper and McNeil 2015, Demonbreun and McNally 2016). The evidence that macromolecular exchange occurs in living cardiomyocytes is based on the detection of albumin (McNeil and Khakee 1992, Clarke et al. 1995) and other extracellular macromolecules (Hoffstein et al. 1975) in the cytoplasm of apparently normal cardiomyocytes. This macromolecular exchange over the plasma membrane is higher if the cardiomyocytes are stressed either by contraction, are stretched by external forces (Page et al. 1992, Swildens et al. 2010), are challenged by beta-adrenergic stimulation (Boutet et al. 1976, Clarke et al. 1995) or after limited ischaemia. The cellular uptake of albumin and other large molecules in these studies seems to occur through transient disruptions in the plasma membrane and is not due to endocytosis or albumin entering T tubules (Clarke et al. 1995). Macromolecular exchange via holes in the plasma membrane without cardiomyocyte death is possible, in part, because the cytoplasm is...
a macromolecular gel with restricted diffusion (Ellis 2001), and because dystrophin complexes stabilize the membrane by forming links between the contracting sarcomere and the extracellular matrix (Allen et al. 2016) (Figure 3(B)).

However, the most important protection against cell death when the plasma membrane is injured is a Ca$^{2+}$-dependent repair system, thoroughly examined by McNeil (Terasaki et al. 1997) and discovered by Heilbrunn almost 90 years ago during his studies of echinoderm oocytes (Heilbrunn 1930). This is why cells do not expel the cytoplasm like a balloon bursting if poked with a micropipette (Terasaki et al. 1997) or if the plasma membrane is injured by a localized laser burn (Demonbreun et al. 2016). Cells are surprisingly resilient and repair membrane holes larger than 10 μm² within seconds, in a process called cell wound repair (Terasaki et al. 1997) (Figure 2(D)). If the same insults are induced in Ca$^{2+}$-free culture media, the membrane holes persist, eventually leading to cell death (McNeil and Kirchhausen 2005). The Ca$^{2+}$ concentration required to elicit a cell wound response is 100 times higher than the Ca$^{2+}$ concentration seen during muscle contraction in most experimental systems (Steinhardt et al. 1994, Terasaki et al. 1997). The result of the cell wound response is the formation of a Ca$^{2+}$-impermeable patch within a few seconds.

The molecular details of cell wound repair are still unclear but most data indicate that the Ca$^{2+}$-impermeable patch is formed by intracellular membrane vesicles fusing with the plasma membrane (Miyake and McNeil 1995, Terasaki et al. 1997, Andrews et al. 2015, Davenport et al. 2016), aided by MG53 (Cai et al. 2009) and dysferlin (Han and Campbell 2007), proteins able to bind to and promote the fusion of two membranes (Figure 2(D)) (McNeil 2014).

Mice (Wenzel et al. 2007) and humans (Han and Campbell 2007) with mutations in genes involved in cell wound repair develop dystrophies, that is contraction-induced accumulation of muscle injury that ultimately leads to muscle weakness. In addition, dystrophies with mutations in other muscle cell-stabilizing functions, such as Duchenne muscular dystrophy, show up to 16-fold upregulation of dysferlin, MG53 and other cell wound repair genes (Waddell et al. 2011). Mice with MG53 mutations show impaired cell wound repair in skeletal muscle (Cai et al. 2009) and are more sensitive to cardiac ischaemia than normal mice (Cao et al. 2010). Dystrophies caused by dysferlin mutations sometimes lead to cardiomyopathy and heart failure in humans (Kuru et al. 2004). Mice with dysferlin mutations display inefficient cell wound repair in cardiomyocytes (Cai et al. 2009) and are more sensitive to stress stimuli (Effertz et al. 2010), likely as a result of cell wound repair with accumulation of extracellular macromolecules in the cytoplasm as the only evidence of transient plasma membrane injury (Boutet et al. 1976). This can be shown by resident colloidal lanthanum in apparently healthy cardiomyocytes surrounding necrotic cardiomyocytes after experimental ischaemic injury and i.v. injection of colloidal lanthanum complexes with a median diameter of 40 Å (Hoffstein et al. 1975, Burton et al. 1977), roughly half the size of albumin.

Under limited ischaemic conditions or limited beta-adrenergic stimulation, elevated serum levels of myocardial injury biomarkers such as LDH, CK and cTn are observed without obvious cell death and sometimes with a reciprocal lowering of these markers in apparently living cardiomyocytes (Chiong et al. 1974, Zhang et al. 2008, Hickman et al. 2010). Although these studies did not detect cardiomyocyte death, it remains possible that these biomarkers are therefore unable to prove that the leakage occurred from viable cardiomyocytes. However, Speiermann group showed that 35% of all LDH can be expelled from resting cardiomyocytes under ischaemic challenge without finding cell death. Taken at face value, this finding would indicate that extensive leakage of the cellular content can result from ischaemically stressed but living cardiomyocytes (Piper et al. 1984, Schwartz et al. 1984). However, how cytoplasmic proteins could escape in such an abundance and still allow the cardiomyocytes to survive is unclear and have, to the best of our knowledge, never been replicated. If the above is correct, then increases in cTn elevation should not be considered proof of cardiomyocyte cell death (Kawahara et al. 2011).

In summary, it is safe to conclude that there are mechanisms that could allow cTn release from living cardiomyocytes. The extent to which cell wounds contribute to the cTn elevations we encounter in the clinic remains to be shown.

**cTn release from skeletal muscle**

The clinical cTn assays all use antibodies that bind epitopes specific to cardiac isoforms of Tnl and TnT. The Roche highsensitive cTnT assay does, however, react with something in skeletal muscle, not yet observed with the cTn assays. This was first observed in patients with neuromuscular disorders and patients with myositis (Botta et al. 2008, Jaffe et al. 2011, Rittoo et al. 2014, Valaperta et al. 2016, Wens et al. 2016).

A cross-reactivity with skeletal muscle extracts from control subjects has also been found. When serum was supplemented *in vitro* with extracts from normal human skeletal muscle such that myoglobin levels were within what can be observed after rhabdomyolysis, cTnT levels, but not cTnI levels, increased above the current cut-off for MI (Schmid et al. 2018).

We (unpublished) and others (Valaperta et al. 2016) have observed a similar reactivity using the Roche high-sensitive cTnT assay in skeletal muscle extracts from healthy humans, pigs and rats, while the Abbott high-sensitive cTnI assay was negative in the same extracts. Therefore, the Roche high-sensitive cTnT assay could in theory become positive in those with chronic skeletal muscle disease or in response to rhabdomyolysis. In these instances, as suggested by the Biomarker...
Group of the European Society of Cardiology (Thygesen et al. 2010), an alternative cTnI assay could be used to check whether the heart is involved, since most of these assays have the same ability to diagnose and exclude myocardial infarction and to define prognosis as the Roche high-sensitive cTnT assay (Shah et al. 2015, Pickering et al. 2016). Whether this strategy it is safe in these conditions has not been examined in a prospective study, but it is in use in several clinics.

**cTn elevation due to decreased cTn clearance**

Once cTnT and cTnI reach the circulation, they are initially cleared with a half-life of 0.5 hours in dogs and rats (Dunn et al. 2011), followed by what we have shown is a slower, apparently kidney-dependent clearance when cTnT drops to concentrations often observed in patients with stable cTnT elevations (Friden et al. 2017) (Figure 6(B)).

It may be that this phenomenon is similar to myoglobin clearance, thoroughly studied by Sylven (Figure 5(A)). At high levels, clearance of radioactively labelled myoglobin injected in healthy controls or in patients with acute MI has a half-life of roughly 1 h, followed by a much slower phase at low myoglobin levels (Sylven 1978, Groth and Sylven 1981) (Figure 5(A)). As myoglobin is a small protein with a molecular weight of 17 kDa, it can pass through the glomerular membrane and is cleared in part by kidney function, as found in patients with rhabdomyolysis and myoglobin-induced acute tubular renal failure.

Despite its ability to pass through the glomerular membrane, clearance of myoglobin at high levels observed after MI or rhabdomyolysis is not influenced by renal function (Wakabayashi et al. 1994, Lappalainen et al. 2002), whereas the low levels found in normal subjects are strongly related to renal function (Hallgren et al. 1978). Myoglobin apparently undergoes predominant extra-renal clearance at high levels (Figure 6(A)) and its renal clearance (Figure 6(B)) becomes apparent only at lower levels.

Myoglobin shares this pharmacokinetic behaviour with low molecular weight heparin cleared by a fast saturable receptor-mediated endocytosis-based system (Boneu et al. 1990) (Figure 6(B)). Early studies have shown that myoglobin accumulates in the liver and spleen (Amako et al. 1963), followed by bilirubinaemia (Bywaters and Beall 1998) in patients who die from massive rhabdomyolysis, indicating that receptor-mediated endocytosis in the reticuloendothelial system is responsible for the extra-renal clearance of myoglobin (Figure 6(A)). This dual clearance system is likely similar to how cTnT is cleared, at least in rats (Figure 5(B)).

For unknown reasons, most of the cTnT measured with clinical high-sensitive cTnT assays are degradation products in patients with stable cTnT elevations (Diris et al. 2003, Friden et al. 2017), and a few hours after the ischaemic event in patients with acute MI (Cardinaels et al. 2013) (Figure 6(C)). The major degradation products are <20 kDa (Cardinaels et al. 2013, Streng et al. 2016, Friden et al. 2017), expected to undergo free passage over the glomerular membrane. Therefore, cTnT measured by the current high-sensitive cTnT assay could be, to some extent, cleared by the kidneys. We have shown this in experiments where we block renal blood flow in rats. In this context, it is important to note that very little radioactive myoglobin reached the urine in Sylven’s subjects mentioned above, likely because tubular resorption prevented myoglobin from reaching the bladder (Sylven 1978). Likewise, tubular resorption of cTn degradation products may explain the fact that, despite apparent renal clearance, cTn levels are low in urine (Ziebig et al. 2003).

Assuming the above, it appears that at low cTnT levels, often found in patients with stable elevations, kidney clearance appears to dominate. Therefore, at steady state and everything else being the same, cTnT levels are roughly twice as high if kidney function is reduced by 50% (Bjurman et al. 2015). As many patients with kidney failure often have stable cTnT levels 10–100 times above the 99th% upper reference limit (Bjurman et al. 2015), other mechanisms must also contribute to the cTnT elevations found in this patient group (Waldum and Os 2013).

However, if the cTnT levels are adjusted for kidney function, the clinical high-sensitive cTnT assay becomes slightly better at distinguishing between patients with and without AMI in the emergency room (Friden et al. 2017). However, cTnT levels adjusted for kidney function do not improve prognostication (Friden et al. 2017).

At the higher cTnT levels often found after large AMIs, extra-renal clearance of cTnT dominates in rats and is not influenced by renal function (Friden et al. 2017), potentially explaining why clearance of cTn is similar among AMI patients with or without renal function (Ellis et al. 2001). The nature of this extra-renal clearance of cTnT has not been

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**Figure 5.** Clearance of myoglobin in humans and cTnT in rats. (A) Concentrations of 125I-labeled myoglobin during 6 hours following i.v. injection in humans. Circles represent 125I levels in serum and triangles represent 125I levels in urine. Dotted lines are projections of the two kinetic profiles found (reproduced from [Sylven 1978] with permission). Most of the 125I in urine did not precipitate in acid, indicating that 125I in the urine was not linked to myoglobin. (B) Relative concentrations of cTnT in rats during 2 hours following i.v. injection of rat cardiac extracts. Dotted lines are projections of two kinetic profiles found (reproduced from [Friden et al. 2017] with permission).

**Figure 6.** Possible clearance mechanisms for cTn discussed in this review (see text for details). (A) Endocytosis (B) Filtration (C) Degradation.
studied but likely occurs via scavenger receptors, a loosely defined group of receptors that are able to bind to a plethora of proteins and other molecules and that direct them to intracellular degradation (Prabhudas et al. 2014) (Figure 6(A)). Scavenger receptor clearance of LDH has been studied by Gruber in rats and rabbits (Smit et al. 1987, Smit et al. 1988). Radioactive LDH is taken up by macrophages (Hayashi and Notkins 1994) in the liver and spleen after i.v. injection. If macrophages are specifically destroyed by the LDH virus, by silica nanoparticles or by other means, mice and monkeys develop stable LDH and CK elevations (Hayashi et al. 1988, Radi et al. 2011), likely due to decreased clearance. An indication that this mechanism may be relevant with regard to stable elevations in humans is a recent finding, showing that stable elevations of CK and LDH correlate with genetic mutations in scavenger receptors (Kristjansson et al. 2016). It is therefore formally possible that stable cTnT elevations could be due to inefficient renal or extra-renal clearance and this is therefore an area of intense study.

Conclusions

Unfortunately, although current knowledge might broaden the perspective, the information in this review makes interpretation of cTn elevations no less complicated. First, because of the possibility that cell wounds could explain some of the release and given the data concerning altered renal clearance, cTn elevations may not be able to serve as direct proof of myocardial necrosis especially in the absence of a clear-cut reason for its release. However, early timely limited cTn increases may be the result of scattered cardiomyocyte necrosis and/or apoptosis, or theoretically even reversible cardiomyocyte injury. Prolonged cTn elevations (>24 h), however, may be due to slow washout from local myocardial injury or the delayed necroptosis following an ischaemic event in both granulation tissue and in the remote myocardium undergoing remodelling.

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